The Influence of Yeast Strain Combinations on the Quality of Sauvignon Blanc Wine

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The main objective of this study was to investigate the influence of different commercial wine yeast strain combinations on wine varietal aroma and glutathione concentration. Four combinations of yeast strains were selected to produce Sauvignon Blanc wine under controlled conditions. The concentration of volatile thiols and methoxypyrazines in produced wines was quantified using GC-MS and the concentration of glutathione during fermentation was monitored and quantified using HPLC-FLD. Sensory analysis of wines was performed by a group of experienced wine assessors. The results indicated that the yeast strain combinations differed significantly in terms of their ability to release volatile thiols and to preserve glutathione levels, but had no impact on methoxypyrazine concentrations. In accordance with the chemical composition of wines, significant differences were found in their sensory quality. It can be concluded that the selection of an appropriate yeast strain combination for alcoholic fermentation presents the potential to greatly modulate wine aroma.

Keywords: wine, yeast strains, volatile thiols, methoxypyrazines, glutathione, aroma

Introduction

The main objective of our study was to investigate the ability of wine yeast strain combinations to enhance wine varietal aroma and, therefore, the sensory quality of Sauvignon Blanc wine. Sauvignon Blanc is an increasingly popular wine variety, cultivated throughout the wine growing regions of the world. The characteristic aroma of Sauvignon Blanc wine can be described as “green” on one hand and “tropical” on the other (Coetzee and du Toit, 2012). Methoxypyrazines are responsible for the “green” flavors of wine, of which 2-methoxy-3-isobutylpyrazine (IBMP) is the most abundant; it is considered to be the main contributor to “vegetative”, “grassy”, “green pepper” and “asparagus” aromas. 2-methoxy-3-isopropylpyrazine (IPMP) contributes to “asparagus” and “earthy” aromas and appears in wines in much lower concentrations. Both methoxypyrazines have a very low perception threshold of around 2 ng L⁻¹ (Marais, 1994). Methoxypyrazines are secondary products of amino acid metabolism in plants and therefore originate directly from the grapes. Their concentration in wine is more affected by climatic and viticultural factors than by oenological ones (Marais, 1998).

Grape is also a source of non-volatile sulfur precursors for 4-methyl-4-sulfanylpentan-2-one (4MSP) (so-called 4-mercapto-4-methylpentan-2-one, 4MMP) and 3-sulfanylhexan-2-one (3SH) (so-called 3-mercaptohexanol, 3MH), which are successfully transformed to volatile, flavor-active thiol compounds during fermentation via the action of yeast β-lyases (Tominaga et al., 1998). Additionally, 3-sulfanylhexyl acetate (3SHA) (so-called 3-mercaptohexyl acetate, 3MHA) is formed by the esterification of 3SH with acetic acid during fermentation via the action of yeast alcohol acetyltransferase (Swiegers et al., 2006). These volatile thiols are extremely potent, having low perception thresholds: 0.8 ng L⁻¹ (4MSP), 60 ng L⁻¹ (3SH), and 4 ng L⁻¹ (3SHA), and they impart “box tree” (4MSP), “passion fruit”, “grapefruit”, “gooseberry”, and “guava” aromas (3SH and 3SHA) (Tominaga et al., 1998; Tominaga et al., 2000). Yeast strains differ in their ability to release 4MSP and 3SH from their precursors and also in their ability to convert 3SH into 3SHA (Howell et al., 2004; Swiegers et al., 2009; King et al., 2010). Yeast strains VIN7, VL3 and VIN13 were shown to release higher quantities of 4MSP and 3SH, while yeast strains QA23 and NT116 showed a high ability to convert 3SH into 3SHA (Swiegers et al., 2009; Dubourdieu et al., 2010).
It was reported that co-inoculations of various *Saccharomyces* yeast strains released more volatile thiols (3SH and 3SHA) than a single yeast strain, indicating the positive effect of co-inoculation (King et al., 2010). It was also shown that some non-*Saccharomyces* yeasts, among others *Torulaspora delbrueckii*, have a high capacity to release 3SH, primarily during the pre-fermentation stage, despite their minimal fermentation rate (Zott et al., 2011).

In contrast to methoxypyrazines, volatile thiols in wine, especially 3SH and 3SHA, are highly oxidizable and extremely reactive with *o*-quinones produced by the oxidation of phenolic compounds (Blanchard et al., 2004). Glutathione, detected mostly in the reduced form (GSH) in grapes, with a mercapto group, has a nucleophilic centre to substitute into the electrophilic ring of *o*-quinone, leading to the formation of 2-S-glutathionyl-caftaric acid or Grape Reaction Product (Singleton et al., 1985). GSH thus plays an important role in protecting the volatile thiols against oxidation, in terms of reacting with *o*-quinone and oxygen first. The concentration of technologically added SO$_2$ in must and wine is therefore important for prevention of GSH oxidation, because it inhibits the action of tyrosinase (Dubernet and Ribéreau-Gayon, 1974) and thus prevents *o*-quinone formation (Jackson, 1994). GSH naturally occurs in grapes and yeasts, and its concentration in wine at the end of fermentation varies depending on the yeast strain used (Lavigne et al., 2007). Typically, the GSH concentration decreases during the first day of fermentation, subsequently rising to reach values of 0.1 – 5.1 mg L$^{-1}$ (Okuda and Yokotsuka, 1999; Park et al., 2000), and at the endpoint of fermentation is usually lower than in the initial must (Lavigne et al., 2007; Du Toit et al., 2007). In addition to its strong antioxidant properties, GSH might be also transported into the yeast cells and involved in yeast sulfur metabolism (Mehdi and Panninckx, 1997; Miyake et al., 1998; Miyake et al., 1999). During the wine maturing on lees, the GSH concentration slightly increases again and is maintained for several months (Lavigne et al., 2007).

Various methods have been used to determine the concentration of methoxypyrazines and volatile thiols in wine. GC-MS methods are most commonly employed (Tominaga and Dubourdieu, 2006; Parr et al., 2007), as they were in our study, but with slight modifications in wine sample preparation. To differentiate sensory quality of wines, various approaches are used. The attribute difference tests measure a single attribute by comparing one sample with another or several others. Depending on the number of wine samples, different tests should be used and a simple ranking test was employed in our study (Meilgaard et al., 1999).

The aim of this study was to evaluate the influence of various commercial wine yeast strains or their combinations on the chemical composition and sensory quality of Sauvignon Blanc wine. Most of the yeast strains chosen were previously shown to be good producers of typical Sauvignon Blanc aroma. In our study, emphasis was placed on the yeasts’ influence on wine varietal aroma and its preservation, and for this purpose, the concentrations of GSH, methoxypyrazines and volatile thiols were determined during vinification. Moreover, wine sensory analysis was conducted using a group of experienced wine assessors.

**Materials and Methods**

**Must handling** Sauvignon Blanc Vitis vinifera (L.) grape must, vintage 2009, was used in this experiment. It was obtained from a winery in the Podravje winegrowing region of Slovenia, after settling with SO$_2$ (50 mg L$^{-1}$) and racking 12 h thereafter. After racking, the concentration of free and total SO$_2$ in must was 15 mg L$^{-1}$ and 62 mg L$^{-1}$, respectively. The must was divided into twelve 3 L carboys. The initial concentrations of specific must compounds were as follows: reducing sugars, 197 g L$^{-1}$; pH, 3.10; total acidity, 5.9 g L$^{-1}$; volatile acidity, 0.01 g L$^{-1}$ and GSH, 6.1 mg L$^{-1}$.

**Yeast strains and fermentation conditions** In the current study, five commercially available yeast strains and their four combinations (Table 1) were used to carry out the alcoholic fermentation. VIN7 plus QA23 (A), Alchemy II (B) and VL3 plus QA23 (C) strains were inoculated only once, whereas

<table>
<thead>
<tr>
<th>Combination</th>
<th>Commercial names of yeast strains</th>
<th>Yeast species</th>
<th>Producer</th>
<th>Dosage (g L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>VIN7</td>
<td><em>S. cerevisiae</em></td>
<td>Anchor Yeast, Cape Town, South Africa</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Lalvin QA23®</td>
<td><em>S. cerevisiae</em></td>
<td>Lallemand S.A., France</td>
<td>0.1</td>
</tr>
<tr>
<td>B</td>
<td>Alchemy II</td>
<td><em>Saccharomyces spp.</em></td>
<td>Anchor Yeast, Cape Town, South Africa</td>
<td>0.2</td>
</tr>
<tr>
<td>C</td>
<td>Zymaflore VL3®</td>
<td><em>S. cerevisiae</em></td>
<td>Laffort, Bordeaux Cedex, France</td>
<td>0.1</td>
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<tr>
<td></td>
<td>Lalvin QA23®</td>
<td><em>S. cerevisiae</em></td>
<td>Lallemand S.A., France</td>
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<td>D</td>
<td>Zymaflore® Alpha Td n. Sacch</td>
<td><em>T. delbrueckii</em></td>
<td>Laffort, Bordeaux Cedex, France</td>
<td>0.1</td>
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<tr>
<td></td>
<td>Zymaflore VL3®</td>
<td><em>S. cerevisiae</em></td>
<td>Laffort, Bordeaux Cedex, France</td>
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young wines were settled with 50 mg L\(^{-1}\) and again after 10 days of fermentation (at reducing sugars concentration 170 g L\(^{-1}\)) with \textit{Saccharomyces} yeast (VL3). Yeast starter culture Alchemy II (B) is a commercial blend of yeast strains formulated to enhance the concentration of volatile thiols in white wines such as Sauvignon Blanc.

No nutrients were added to the must during alcoholic fermentation. Fermentations (A-D) were carried out in triplicate at controlled room temperature \(15^\circ\text{C}\). The fermentation was monitored by weighing the carboys and measuring the amount of exhausted CO\(_2\). After 36 days of fermentation, the young wines were settled with 50 mg L\(^{-1}\) SO\(_2\) at low temperature \(4^\circ\text{C}\). The wines were racked and bottled 1 week after the end of alcoholic fermentation. The basic chemical parameters (reducing sugars, alcohol, pH, total acidity and volatile acidity) were determined immediately in the bottled wines. The concentration of GSH was determined at various stages of vinification: in must prior to inoculation, 1 h after inoculation, the ninth day of alcoholic fermentation (in the exponential phase of yeast starter cultures A, B and C or in the stationary phase of \textit{T. delbrueckii} of yeast starter culture D), and at the end of alcoholic fermentation. Volatile thiols (4MSP, 3SH and 3SHA) were determined 1 month after bottling and methoxyypyrazines (IBMP, IPMP) were determined immediately after the completion of alcoholic fermentation.

\textbf{Determination of chemical parameters in must and wine}

For determination of basic chemical parameters, reducing sugars, alcohol, total acidity as tartaric acid, volatile acidity as acetic acid, and pH, the reference methods of the European Union (EEC 1990) were used.

\textbf{Determination of glutathione concentration in must and wine}

GSH determination was performed using the method described in Janeš \textit{et al.} (2010). The samples of must and wine were mixed with methanol at a 1:10 ratio to avoid oxidation of GSH. An Agilent 1200 Series HPLC with fluorescence detector and high-performance auto sampler for online derivatization, controlled by Agilent Chemstation Rev. B.03.01 (Agilent Technologies, Palo Alto, CA, USA), was used for GSH detection and quantification. The on-line pre-column derivatization with \(o\)-phthalaldehyde and 2-aminoethanol was used for each sample just before injection. Separation was performed at 25°C using a Synergi Fusion-RP 80A column (4 \(\mu\)m, 150 mm \(\times\) 2.0 mm i.d.) from Phenomenex (Torrance, CA, USA). The mobile phase consisted of 50 mM sodium acetate buffer at pH 5.7 (Solvent A) and methanol (Solvent B). The gradient was as follows: isocratic at 10% Solvent B for 1 min; 10% to 34% Solvent B in 26 min; 34% to 100% Solvent B in 2 min; isocratic at 100% Solvent B for 3 min; 100% to 10% Solvent B in 2 min; 5 min conditioning at 10% Solvent B. The column was equilibrated 5 min prior to the next analysis. The flow rate was 0.7 mL min\(^{-1}\) and the injection volume was 9 \(\mu\)L. The excitation wavelength was 340 nm and 450 nm for emission. A nine-point calibration curve for this method was linear in the range of 0.2 mg L\(^{-1}\) to 60 mg L\(^{-1}\); \(R^2 = 0.9984\). Limit of detection (LOD) was 0.06 mg L\(^{-1}\) and limit of quantification (LOQ) was 0.2 mg L\(^{-1}\).

\textbf{Determination of volatile thiols in wine}

Determination of volatile thiols (4MSP, 3SH and 3SHA) in bottled wines was performed using the slightly modified method described by Tominaga and Dubourdieu (2006). First, the Dowex gel was reactivated by flushing with 0.1 M HCl, and then rinsed with Milli-Q water to adjust the pH to 5 – 6. The Dowex gel was then transferred to glass extraction columns and rinsed again with 100 mL of Milli-Q water at a flow rate of a drop per 3 s. The wine sample (50 mL) was prepared with the addition of 2 mM \(p\)-hydroxymercuroibenzoate and 0.02 mM tert-butyl-4-methoxyphenol (butylated hydroxyanisole, BHA). The sample was stirred for 1 min, then the internal standards 4-methoxy-2-methyl-2-sulfanylbutane (4M2M2SB), \([^{2}\text{H}_2]\)-3-sulfanylhexyl acetate (d3SHA) and \([^{2}\text{H}_2]\)-3-sulfanylhexan-1-ol (d3SH) were added and stirred for an additional 10 min. The pH of the wine sample was adjusted to 7 and the prepared sample was transferred to the extraction column at a flow rate of a drop per 5 s. The column was washed with wash buffer (0.1 M sodium acetate with BHA addition, pH 6). The orientation of the column was moved 180° and the column was washed in reverse order with cysteine buffer (0.1 M sodium acetate with addition of cysteine and BHA, pH 6). The flow rate of cysteine buffer was a drop per 7 s. Ethyl acetate and dichloromethane were added to the eluate and stirred for 5 min. The eluate was transferred to a separatory funnel and thoroughly stirred, and the organic phase was captured in a 20-mL vial filled with sodium sulfate, which bound the water in the sample. The water-free organic fraction was filtered through glass wool into a 50-mL conical flask and evaporated at 250 mbar in a water bath (20°C) to a volume of 0.5 mL. Further evaporation was carried out in a 2-mL vial at 100 mbar in a water bath (20°C) to a volume of 30 \(\mu\)L.

Thiols were identified and quantified with a gas chromatograph (Agilent Technologies 7890A) equipped with a MPS 2 automatic sampler (Gerstel, Mühlheim an der Ruhr, Germany) coupled with a mass spectrometric detector (Agilent Technologies 5975C upgraded with Triple Axis detector). The chromatograph was equipped with a capillary column (Agilent J&W GC column: HP-INNOWAX, 60 m \(\times\) 0.25 mm; film thickness 0.25 \(\mu\)m). Helium gas at a constant flow of 0.6 mL min\(^{-1}\) was used as a carrier. The injector temperature was set to 240°C with an oven temperature gradient of 50°C for 5 min, then from 50°C to 115°C at 3°C min\(^{-1}\), then
from 115°C to 150°C at 40°C min⁻¹, 3 min at 150°C, then from 150°C to 205°C at 3°C min⁻¹, from 205°C to 250°C at 10°C min⁻¹, 19.625 min at 250°C, then back from 250°C to 50°C at 40°C min⁻¹ and 3 min at 50°C. The ion source temperature was 230°C, the auxiliary temperature was 250°C and the quadrupole temperature was 150°C. For qualitative determination, retention time and mass spectrum in Selective Ion Monitoring mode (SIM) were used. For IPMP, the target ion was 137 m/z and the qualifier 152 m/z. For [²H₃]-IBMP, the target ion was 127 m/z and the qualifier 154 m/z. For IBMP, the target ion was 124 m/z and the qualifier 151 m/z.

Calibration was performed using calibration standards in alcoholic solution. First, the preparation of standards was performed using IBMP, [²H₃]-IBMP and IPMP (Sigma Aldrich, St. Louis, MO, USA). All standards were of 99% purity. Stock solution was prepared in methanol (Sigma Aldrich) with 250 mg L⁻¹ IBMP, 500 mg L⁻¹ [²H₃]-IBMP and 280 mg L⁻¹ IPMP. Working solutions were prepared in methanol with 2.5 μg L⁻¹ IBMP, 5.0 μg L⁻¹ [²H₃]-IBMP and 2.8 μg L⁻¹ IPMP. Alcoholic solution was prepared in a 1000-mL flask with 500 mL of Milli Q water, 120 mL of absolute ethanol (Sigma Aldrich) and 1 g of tartaric acid; the flask was filled up to the mark with water (Milli Q) and the pH adjusted to 3.2. The calibration standards were prepared in 25-mL flasks with alcoholic solution and the addition of 250 μL of IBMP working solution, 250 μL of IPMP working solution and 125 μL of [²H₃]-IBMP working solution. In a 20-mL SPME vial, 1.6 mL of calibration standard, 6.4 mL of Milli-Q water and 2 mL of 4 M NaOH were pipetted, then 3 g of NaCl and a stir bar were added. Closed vials were placed on a magnetic stirrer to dissolve the NaCl.

Nine-point calibration (four repetitions per calibration level) was performed for the deployed method using spiked alcoholic solutions. The calibration curve was linear in the range from 1 ng L⁻¹ to 196 ng L⁻¹ for IBMP and from 1 ng L⁻¹ to 200 ng L⁻¹ for IPMP; R² was 0.9986 for IBMP and 0.9985 for IPMP. The limit of detection (LOD) was 0.4 ng L⁻¹ for IBMP and 0.5 ng L⁻¹ for IPMP; the limit of quantification (LOQ) was 1.2 ng L⁻¹ for IBMP and 1.6 ng L⁻¹ for IPMP. Multiple linear regression (F-test) was used for linearity and range determination and a calibration curve was used to calculate the LOD and LOQ.

**Sensory descriptive analysis** After 6 months of wine maturation at 12°C, the replicates of wines fermented with each yeast starter culture were blended, and sensory evaluation was performed using a group of 12 experienced wine assessors. Wines were served at 12°C. A ranking test was used for the attributes tropical fruits flavor and overall wine quality. Each assessor evaluated a series of four wines for each of the two attributes. The order of each series was randomly selected for each assessor. Four wines represented the different combinations of yeast strains used to conduct alcoholic fermentation (A-D). Assessors ranked the wines from best (Grade 4) to inferior (Grade 1) for each of the attributes.
**Statistical analysis**  The quantitative analytical data was analyzed for statistical significance using an analysis of variance (ANOVA) and individual comparisons with the LSD test using Statgraphics® Centurion XVI statistical software package (StatPoint Technologies, Warenton, VA, USA). The results of sensory evaluation were statistically analyzed using the Friedman analysis (Meilgaard et al., 1999).

**Results**

**Fermentation kinetics**  The fermentation kinetics (data not shown) significantly differed depending on the yeast starter culture. At the beginning of fermentation, no significant differences in exhausted CO₂ per liter of fermenting must were evident, with the exception of yeast starter culture C, which exhibited the fastest fermentation kinetics for the 3 days following inoculation (22 g CO₂ L⁻¹). By the ninth day, significant differences in fermentation kinetics amongst all yeast starter cultures were found; the fastest fermentation kinetics were found within yeast starter culture C (54 g CO₂ L⁻¹), followed by the yeast starter cultures B (46 g CO₂ L⁻¹) and A (39 g CO₂ L⁻¹). Yeast starter culture D showed the weakest fermentation kinetics (14 g CO₂ L⁻¹). In the following days of fermentation, the yeast starter culture B showed the fastest fermentation kinetics, while culture D was the slowest. After the 16 day and up to the endpoint of fermentation, yeast starter culture C showed the slowest fermentation kinetics and yeast starter culture A the fastest. However, at the end of fermentation (36 days) the amount of exhausted CO₂ was comparable between all investigated yeast starter cultures, ranging from 82 to 92 g CO₂ L⁻¹.

**Chemical composition of wine**  As shown in Table 2, there were no significant differences between wines in the concentration of reducing sugars, which ranged from 1.37 g L⁻¹ (C) to 1.93 g L⁻¹ (A), and ethanol concentration, which ranged from 11.1 vol.% (D) to 11.4 vol.% (B, C). Significant differences were observed in total acidity concentration, of which the highest were determined in wines A (6.60 g L⁻¹) and C (6.53 g L⁻¹), and the lowest in wine B (6.33 g L⁻¹). With respect to concentration of volatile acidity, only wine A, with the highest concentration (0.79 g L⁻¹), differed significantly from the other wines (0.42 g L⁻¹ (B) to 0.56 g L⁻¹ (C, D)). Also, the pH value in wines A (3.28) and B (3.27) was significantly higher than in wines C (3.24) and D (3.23).

**Glutathione concentration during and after alcoholic fermentation**  The concentration of GSH 1 h after the inoculation of must with yeast starter cultures was lower than in the initial must (6.1 mg L⁻¹) in all cases, with no significant differences between wines (5.1 mg L⁻¹ (C) to 5.5 mg L⁻¹ (A)) (Figure 1). During the exponential phase of yeast growth
(the ninth day of fermentation), the GSH concentration increased to greater than that in must, with the exception of the sample fermenting with yeast starter culture D, in which the concentration decreased (1.9 mg L⁻¹). In this phase, significantly higher GSH concentrations were determined in samples fermented with yeast starter cultures B (7.8 mg L⁻¹) and C (7.7 mg L⁻¹). At the end of alcoholic fermentation, the GSH concentration decreased in all cases with the exception of wine D, which showed an increase in GSH concentration. The highest significant GSH levels were observed in young wine A (5.0 mg L⁻¹), followed by the wines B (3.9 mg L⁻¹) and D (3.3 mg L⁻¹). The lowest GSH concentration was observed in wine C (2.4 mg L⁻¹).

Concentration of methoxypyrazines in wines after alcoholic fermentation As shown in Table 2, there were no significant differences between wines in concentration of methoxypyrazine IBMP, which ranged from 3.08 ng L⁻¹ (A) to 3.78 ng L⁻¹ (D). The concentrations of methoxypyrazine IPMP in the wines were lower than the LOQ (1.6 ng L⁻¹).

Concentration of volatile thiols in wines after alcoholic fermentation The concentration of volatile thiols in wines was significantly dependent on the yeast starter culture used for fermentation (Figure 2). The highest significant level of 4MSP was observed in wine B (5.6 ng L⁻¹), followed by wines A (4.7 ng L⁻¹) and C (3.9 ng L⁻¹). The lowest significant level was determined in wine D (2.3 ng L⁻¹). The concentration of 3SH was significantly lower in wine D (2.3 ng L⁻¹), while the concentration of 3SH in other wines ranged from 1053.2 ng L⁻¹ (C) to 1125.8 ng L⁻¹ (D). Significant differences in 3SHA concentration were also found between wines; levels were significantly higher in wines A (154.9 ng L⁻¹) and D (142.8 ng L⁻¹), followed by wine B (100.1 ng L⁻¹). The lowest significant level of 3SHA was determined in wine C (54.2 ng L⁻¹). To calculate the conversion rate of 3SH into 3SHA by different yeast starter cultures, the concentration of 3SHA was divided by the concentration of 3SH (Swiegers et al., 2009). The yeast starter culture A had the highest significant conversion rate (21%), followed by yeast starter cultures D (13%) and B (9%). The lowest significant conversion rate was observed for the yeast starter culture C (5%).

Sensory analysis Results of the sensory evaluation are presented in Table 3. For the sensory attribute flavor of tropical fruits, wine B was ranked the highest (38 points), followed by wines C (34 points) and A (26 points). Wine D, with 22 points, was rated significantly lower (by 16 points) than wine B. For the sensory attribute overall wine quality, wine B was again ranked the highest (34 points), followed by wines C (33 points), D (27 points) and A (26 points). Differences in overall wine quality were not significant.

Discussion
As previously reported, non-Saccharomyces wine yeasts can influence the fermentation kinetics of a mixed starter culture with Saccharomyces spp. (Ciani et al., 2006). Our results confirmed this observation; T. delbrueckii, in the mixed starter culture D, showed a reduced fermentation rate during the first 10 days of the process, compared to the pure cultures of Saccharomyces yeasts (A-C). However, after inoculation with the Saccharomyces yeasts of starter culture D,
the fermentation kinetics improved and the final amount of exhausted CO₂ was comparable between all investigated fermentations. The longer fermentation period might be due to a lack of nutrients in the must, since the concentration of free amino acid nitrogen was rather low (115.5 mg N L⁻¹) and no nutrients were added. The highest significant volatile acidity of wine A might be due to the slowest fermentation rate at the beginning or as a result of the action of the VIN7 yeast strain, which has previously been shown to produce large amounts of volatile acidity (Swiegers et al., 2009).

Our study showed that the decrease in GSH concentration of fermenting must at the beginning of fermentation was minimal, while in later fermentation stages the concentration of GSH varies significantly, depending on the yeast starter culture used. The increase in GSH concentration observed during fermentation has also been shown by other authors and might be due to the release of yeast intracellular GSH during autolysis (Park et al., 2000; Lavigne et al., 2007). However, in the case of wine D, the concentration of GSH decreased significantly during fermentation, indicating either an extensive uptake of GSH by T. delbrueckii as shown for S. cerevisiae (Miyake et al., 1998), or an early oxidation of GSH due to reduced fermentation rate. The highest GSH concentration was observed in wine A at the end of fermentation and might be attributed to the yeast starter cultures’ fermentation rate (relatively constant and fast towards the end). In contrast, the yeast starter culture C showed a very fast fermentation rate at the beginning of fermentation, and the slowest rate towards the end, which might lead to an early oxidation of the wine and consequently the lowest GSH concentration. It can be concluded that yeast strain combination and, consequently, fermentation kinetics strongly influence the final concentration of GSH in wine. Additionally, the concentration of GSH in finished wines was lower than in the initial must, as reported by others (Lavigne et al., 2007; Du Toit et al., 2007), which is probably a consequence of yeast GSH uptake (Miyake et al., 1998), induced by nitrogen or sulfur limitation, and its use as a major sulfur reserve compound (Mehdi and Panninckx, 1997; Miyake et al., 1999) It was expected that higher concentrations of GSH would contribute to enhanced sensory quality of wines due to reduced wine oxidation. However, GSH concentrations were probably too low (3.7 mg L⁻¹ on average, which is lower than that obtained by Janeš et al., (2010) (12 mg L⁻¹ on average)) to correlate with the flavor of wine.

In our study, different yeast starter cultures did not influence the methoxypyrazine concentration, since there were no significant differences found in their concentration between wines. On the other hand, we showed that the concentration of volatile thiols differed significantly depending on the yeast starter culture used, as was previously reported by others (Dubourdieu et al., 2006; Swiegers et al., 2009). While Saccharomyces yeast starter cultures (VIN7 plus QA23, Alchemy II and VL3 plus QA23) showed a relatively high capacity to release 4MSP, the yeast starter culture VL3 plus T. delbrueckii showed relatively poor capacity, since wine D contained significantly lower concentrations of the volatile thiol. In addition, wines A, B and C were ranked higher than wine D for the sensory attribute flavor of tropical fruits. Significantly lower concentrations of 3SH in wines fermented with yeast starter culture VIN7 plus QA23 might be a result of the high capacity of this starter culture to convert 3SH into 3SHA, which was already confirmed for both yeast strains by Swiegers et al., 2009. While co-inoculation with yeast strains VL3 and QA23 (C) resulted in the lowest 3SHA concentrations in wine, co-inoculation with yeast strains VL3 and T. delbrueckii (D), on the other hand, resulted in high 3SH and 3SHA concentrations. The assumption could be made that co-inoculation of yeast strains VL3 and QA23 might have a negative impact on 3SHA concentration in wine. Despite higher concentrations of 3SHA, wines A and D were ranked the lowest for flavor of tropical fruits and overall quality, while wines B and C, with high concentrations of 4MSP (B) and 3SH and lower concentrations of 3SHA, were ranked highest for both sensory attributes. Despite the significant differences in 3SHA concentration in the produced wines, it appears that differences in 3SH concentration played a more important role in the perception of tropical flavor by the sensory panel. In addition, the concentrations of 3SHA were much lower than observed in some New Zealand Sauvignon Blanc wines (Herbst-Johnstone et al., 2011) and its influence on tropical flavor was probably less obvious.

The present results indicate that the selection of an appropriate yeast starter culture for the fermentation of Sauvignon Blanc must significantly influences the concentrations of GSH and volatile thiols, and therefore the sensory quality of wine. Methoxypyrazine concentration was not influenced by the yeast strains. Regarding the sensory perception of flavor of tropical fruits and the overall wine quality, the most appropriate yeast starter culture for fermentation of Sauvignon Blanc must under the investigated conditions was Alchemy II, which exhibited a high capacity to release 4MSP and 3SH from their precursors. A similar capacity was observed with the yeast starter culture VL3 plus QA23, which produced the wine ranked second. Yeast starter cultures VIN7 plus QA23 and VL3 plus T. delbrueckii were, in this aspect, shown to be less appropriate for the production of Sauvignon Blanc wine, despite the higher capacity to convert 3SH into 3SHA.

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